Gametogenesis of the Gall-Fly, Neuroterus lenticularis.—Part II.

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[Plate 17.]

Introduction.

In the first part of this paper* I described the life-history, spermatogenesis, maturation of the fertilised egg, fertilisation and segmentation divisions of the summer generation of the gall-fly *Neuroterus lenticularis* (*Spathegaster baccarum*), and gave some observations on the maturation of the eggs of the spring generation, and on the somatic mitoses in males and females. These latter observations were admittedly incomplete owing to lack of material, and this second part of the paper attempts to complete the account of what was left uncertain in the first communication. Before proceeding to describe my fresh observations it may be well shortly to summarise the results arrived at in the first part.

The spring generation of *N. lenticularis* consists of females which lay parthenogenetic eggs; those laid by some females develop into males, those laid by others develop into sexual females, both of which hatch in June. The eggs of the summer females undergo a double maturation division, leaving 10 chromosomes in the nucleus of the mature egg. The spermatogonia of the males in the same generation have 10 chromosomes; the first spermatocyte division is suppressed; in the second spermatocyte division the chromosomes divide, 10 entering each spermatid. A small staining extra-nuclear body found in the spermatocytes does not divide, and is included in only half of the spermatids. In fertilisation, the egg and sperm nuclei each contain 10 chromosomes, and the segmentation divisions of the fertilised egg show 20. For a complete study of the maturation and segmentation of the parthenogenetic spring eggs my material was insufficient, but it suggested that the eggs laid by some females underwent maturation and showed 10 chromosomes in the segmentation divisions, and that others had no polar divisions and showed 20. Since somatic and oogonial mitoses of the summer female have 20 chromosomes, but the spermatogonial mitoses and those of the developing nervous system in the male only 10, it was suggested that the eggs which undergo maturation

* 'Roy. Soc. Proc.,' B, vol. 82, p. 88.

produce males, the others females. An attempt was made, in conclusion, to connect these phenomena with what is known of sex-determination in other animals.

The material on which the observations here recorded were made was collected in March and April, 1910; the methods adopted were essentially those described in Part I, the eggs laid by each female being fixed and preserved separately at intervals from one to nine hours or more after being laid.

In addition to Petrunkewitsch's fluid another formula of alcoholic sublimate was tried (50 per cent. alcohol with sublimate to saturation + 6 per cent. acetic acid), but the results obtained were inferior to those yielded by Petrunkewitsch's mixture. Additional experiments were made to test the conclusion that the eggs laid by any fly are all male-producing or all female-producing; 65 flies were sleeved out in March and April on oak-trees which had proved successful in the previous year, and 16 of these yielded galls early in June. Unfortunately many of the galls had been attacked by parasites, greatly reducing the number of flies that hatched, so that the results, although not discordant with the last year's experiments, are of little value. The only considerable number obtained was 12 females from 19 galls produced by one parent. Mr. H. Scott has very kindly examined the external characters and mouth parts of a few male-producing and female-producing parthenogenetic females, and finds no recognisable differences between them except slight differences in size, which are probably accidental.

Maturation of the Eggs of the Spring (Parthenogenetic) Generation.

The part of the previous work which chiefly required completion and confirmation was that dealing with the maturation of the spring (parthenogenetic) eggs. It was found that in the eggs laid by some females polar divisions, or, at a later stage, groups of polar chromosomes, occurred, and, in the few cases in which the chromosomes of the segmentation mitoses could be counted in such eggs, the number appeared to be 10. In eggs laid by other females no polar chromosomes could be found, and these eggs showed 20 chromosomes where they could be counted in the segmentation division-figures. The number of cases observed was, however, not sufficient to substantiate this conclusion with certainty, especially since in some of the less well-preserved eggs the chromosomes were clumped together in the metaphase of the segmentation divisions, and might show in one part of the egg what appeared to be 10 or even fewer large chromosomes,

while elsewhere in the same egg the full diploid number (20) was clearly present.

In the spring of 1910 I collected series of eggs from a large number of females, and from them obtained nineteen series of sections of well-preserved eggs, averaging about 27 eggs from each female, besides several smaller or less well-preserved series. Since the rate of development had proved rather variable in previous years, these were fixed at various ages, and not all the series show every stage from the undivided egg-nucleus up to segmentation divisions in which the chromosomes can be counted. Since, however, most of the series included eggs between two and six hours old, the majority show most of the stages desired, and several series are very complete. A comparison of the various series shows at once that they mostly fall into two groups: some show the diploid number (20) of chromosomes whenever the segmentation mitoses are clearly countable, and these never contain groups of polar chromosomes in the segmentation stages; others show the haploid number (10) in segmentation divisions, and in these a double or triple group of polar chromosomes can be found at the edge of the egg in nearly every specimen in which the sections are complete. Out of the nineteen series of eggs, five series are of the first type, containing diploid segmentation divisions and no polar chromosomes, while one other series, though containing only one egg with a recognisable mitosis, belongs to this type from the complete absence of polar chromosomes in the early segmentation stages. Of the second (haploid) type, seven series of eggs show undoubted polar mitoses or polar chromosomes; in two of these segmentation mitoses occur which are certainly haploid, and in two others, though the chromosomes cannot be counted accurately, the mitoses are almost certainly haploid. In the other three series there are no segmentation mitoses at a stage when the number of chromosomes can be estimated. Judged by the presence or absence of polar chromosomes, therefore, there are six series of the unreduced and seven of the reduced type; five of the former show diploid, two of the latter certainly haploid, segmentation mitoses. In the series of eggs collected in 1909 the proportion of the diploid type was probably greater. In the remaining six of the 1910 series all the eggs are too young to be classified with confidence. The fact that only two out of seven haploid series contain eggs with undoubted haploid segmentation mitoses, may seem insufficient evidence that eggs containing polar chromosomes have reduced segmentation nuclei, but it must be remembered: (1) that in none of the eggs with diploid mitoses can polar chromosomes be found, and (2) that every egg in which the sections are all complete shows polar chromosomes when the mitoses are haploid. In some

of these the reduced number is so clear as to leave no doubt, while in a number of eggs of the diploid series the absence of polar chromosomes, even at a stage when there are only two segmentation nuclei, is equally certain. This will become clearer in the detailed account of the maturation and segmentation stages which follows.

In eggs belonging to both diploid and haploid series the early stages are alike. The nucleus appears as a rather small oval body, deeply and evenly stained, usually about half-way between the free end of the egg and the base of the stalk, either in the centre of the egg or nearer one side (Plate 17, fig. 1). It then approaches one side of the egg, at first with its long axis perpendicular to the egg-membrane, but as it reaches the edge it becomes round and finally flattened against the edge (figs. 2, 3). The nucleus then swells, and becomes an oval vesicle lying close to the edge with its long axis parallel to it, and an irregular spireme or network of chromatin bodies is now visible within it (figs. 4, 5). From this stage onward the behaviour of the nucleus in eggs of the two types is different. In eggs of the diploid series the nucleus in prophase of division as just described sinks for a short distance into the egg, and forms a mitotic figure parallel with the edge of the egg. I have only one egg in which the equatorial plate of this division is shown, and since it is lying sideways to the plane of the section the chromosomes cannot be counted accurately, but they are almost certainly more than ten. Several anaphase stages with the axis of the spindle parallel with the edge of the egg have been found: in one of these (fig. 6, *a*, *b*), in which the figure is cut in face in two successive sections, in one section 10 chromosomes are visible, while at the other a larger number appears, but it is not clear how many are cut so as to appear in both sections. Many cases have been found in which the first division is just completed, and two vesicular nuclei occur, both near the edge of the egg and lying in a line parallel with it. At first the nuclei are near together and are quite small (fig. 7); later they enlarge and are found at a greater distance apart. In several such eggs the series of sections is quite complete, and no trace of polar chromosomes can be found. The next division of these two nuclei appears usually to be perpendicular to the edge of the egg, and may be seen to show considerably over 10 chromosomes, even if they cannot be counted with complete accuracy. In the later divisions the 20 chromosomes can usually be counted most easily in anaphase (fig. 8, *cf.* fig. 46 of Part I of this paper); in some cases in prophase about 20 irregular meridional chromatic bands can be counted in the nucleus before the membrane disappears (fig. 9). In some later prophases the intra-nuclear origin of the greater part of the spindle is seen (fig. 10). Sometimes in metaphase in eggs which are not very well fixed the chromosomes become

clumped together, and may give the appearance of about 10 or fewer very thick chromosomes. If care is not exercised such figures may be mistaken for haploid mitoses, but a little experience at once shows the difference, and usually in other parts of these eggs undoubted diploid mitoses may be found.

In all these cases no polar chromosomes can be found in any egg of the series to which they belong.

In the eggs of the haploid type the behaviour of the nucleus does not differ from that described until it has become flattened against the edge of the egg, and has then begun to swell, so that instead of being an evenly stained body it takes the form of an oval vesicle in which chromatic masses are distinguishable. Instead of sinking into the egg it continues to enlarge at the edge and occasionally a stage may be found in which the chromatin is aggregated at the two sides of the nucleus, one mass being next to the edge of the egg and the other towards the inside (fig. 11). As was described in the previous part, both for the summer and spring eggs, no regular equatorial plate has ever been found. The next stage, of which I have a number of examples, shows the chromosomes separating into two groups; an inner in which they appear as a group of parallel rods as in an ordinary anaphase, and an outer in which they are very irregularly arranged in a loose group close to the edge of the egg.

In some sections of this stage (fig. 12) it can be seen that the inner group of parallel chromosomes is in the equator of an elongated mitotic spindle, and although I have no very clear figures of these stages, those which I have suggest that the rod-like chromosomes divide transversely, giving rise at the inner end of the spindle to the egg-nucleus, at the outer end to a second group of polar chromosomes (fig. 13, *a*, *b*, *cf.* Plate 3, fig. 42 of first part of this paper).

In this way a large outer and smaller inner group of polar chromosomes arise, and during the segmentation stages two groups are often found, one of which frequently contains about 10 chromosomes. In other cases the outer group appears to divide into two, both of which are irregular and can never be counted with accuracy. Fig. 14, *a*, *b*, represents the only case in which I have found this division taking place. On the whole it is clear that the polar divisions of the spring eggs which undergo maturation do not differ widely from those of the summer eggs, which require fertilisation, as described in Part I. There is in both essentially a double polar division, in the first part of which an irregular outer group of chromosomes separates from a more regular inner group, followed immediately by the division of the inner into egg-nucleus and inner polar group, each of which contains the

reduced number of chromosomes. The whole process is so nearly simultaneous as to give the impression of a single division, a fact which led me to hazard the suggestion in Part I of this paper that only one maturation occurred in the parthenogenetic eggs. The fresh material since collected, however, shows that the inner chromosome group does undoubtedly divide just after its separation from the outer, and the phenomena must therefore be interpreted, as in the summer eggs, as a modified double polar division. The maturation thus differs rather in detail than in essence from what occurs in all other Hymenoptera in which the polar divisions have been examined.

After the polar divisions, the egg-nucleus sinks into the yolk and almost immediately forms the first segmentation spindle, so that the prophase of this mitosis may not rarely be found in the same section with the polar chromosomes. The axis of the first segmentation spindle appears usually to lie nearly, but not quite, parallel to the long axis of the egg; after the division is completed, the daughter nuclei travel widely apart, so that the division-figures of the second segmentation mitoses are usually widely separated. In these and the later segmentation divisions the chromosomes are most easily counted in anaphase; in metaphase they may be clumped, but, even so, differ from the clumped diploid groups described above in their smaller size. In anaphase figures cut across it is possible to count 10 with confidence in some cases (fig. 15), and in a number of others to see clearly that the number is undoubtedly much less than 20, though not accurately countable (figs. 16, *a*, *b*; 17, *a*, *b*). In a few cases one chromosome appears to lag very much behind the others, as if it was being left out of the nucleus altogether, but in other eggs I have not seen anything of the kind, even when mitoses of the same stage are present. During the segmentation mitoses, the groups of polar chromosomes persist at the edge of the egg (fig. 18), but when the nuclei come to the surface to form the blastoderm, they are no longer recognisable. The chromosomes and mitotic figures in the blastoderm divisions are too small and crowded for counting to be possible.

Later Stages: Segmentation and Blastoderm.

I have no great number of sections of eggs in the later segmentation and blastoderm stages, but among them are some which show phenomena of interest. In eggs which have a well-developed blastoderm, a number of nuclei remain in the yolk, and these are often of two types in the same egg. There are a number of rather large vesicular nuclei, sometimes showing a faint reticulum, and, in the others, a clear space inside the membrane enclosing a more compact stained mass, rather suggesting that the nuclear contents have contracted. Among these there are frequently many smaller

nuclei, either evenly stained throughout or consisting of a deeply stained mass surrounded by a narrow clear space. When the nuclei of the blastoderm are dividing, which they all do simultaneously, they show clearly defined, though very small and compact, mitotic spindles; the nuclei of the yolk in the same egg show no spindles, and strongly suggest amitotic division, for various stages can be found, from nuclei which are simply elongated to two or sometimes three nuclei in which the membranes are in contact (figs. 19, 20, *a*, *b*). Stages of this apparent amitotic division are found in the yolk-nuclei of most eggs in the blastoderm stage, whether the blastoderm-nuclei are undergoing division or not. In advanced blastoderm stages below the layer of blastoderm-nuclei there is a belt of protoplasm with no yolk, and inside this the yolk fills the centre of the egg, and contains all the yolk-nuclei, none being found in the protoplasmic belt.

In two large series of eggs (each laid by a single female) a number of later segmentation stages are represented preceding the formation of the blastoderm. Unfortunately, neither of these series contains any egg which shows conclusively whether it is haploid or diploid. Both contain eggs showing bodies at the edge which might be remnants of the polar chromosomes, but which cannot be identified as such with certainty. In some eggs, but not in all, of both series, very large nuclei occur, among which are scattered others of the normal size. In late segmentation stages these large nuclei are sometimes found in division; the figures are obscure, possibly owing to imperfect fixation, but appear to be diploid. Divisions of the smaller nuclei, on the other hand, suggest haploid figures, but I have none in which the chromosomes can be counted (fig. 21, *a*, *b*). In other eggs, some of these large nuclei are either undergoing amitotic division or are being formed by fusion of two or more smaller nuclei; groups of two, three, or even more nuclei are found in close contact, while here and there are large, apparently single nuclei as big as the group of smaller ones (figs. 22, 23, 24). In some eggs among these are nuclei which are drawn out into processes, or have an irregular shape (fig. 25). At this stage the distinction between yolk- and blastoderm-nuclei cannot be made, for all are scattered indiscriminately through the egg; they are most numerous at the lower end of the egg, towards the stalk. In eggs of other series the nuclei are all alike at this stage, and show nothing unusual, but that the two series described are not entirely exceptional is shown by the occurrence of one or two similar eggs among later segmentation stages collected in 1908. That the phenomena are due entirely to bad preservation appears improbable. From incomplete observations such as these it would be premature to draw definite conclusions, but it may be suggested provisionally that during the later

segmentation stages the nuclei become differentiated into two kinds; some will form the blastoderm and continue to divide normally by mitosis, others remain as yolk-nuclei and show signs of degeneration in the frequent massing together of their chromatin into a single, large, deeply-staining mass, in their great variation in size, possibly due to fusion of nuclei in some cases, and lastly, in dividing irregularly by amitosis. I find that such "yolk-nuclei" exist also in the blastoderm stages of the summer (fertilised) eggs, and these show signs of degeneration and indications of amitosis similar to those found in the spring eggs. In an embryo of the summer generation fixed when three days old, the yolk is already largely absorbed and the yolk-nuclei appear to be undergoing atrophy. Whether these suggestions are correct can only be determined by further work on fresh material collected for the purpose.

Ovarian Mitoses of Parthenogenetic Females.

In the spermatocyte divisions of the male, I described (Part I, pp. 92, 94, and 95) a small extra-nuclear body which does not divide with the cell, and is, therefore, present in only half the spermatids. It seemed not impossible that the presence of this body in half the spermatozoa might determine whether the fertilised egg developed into a male-producing or female-producing parthenogenetic individual. To recognise such a body in the fully grown egg would be almost impossible, owing to the quantity of yolk, but it seemed just possible that it might occur in the oogonial mitoses of some of the parthenogenetic females. I have cut sections of the ovaries of seven larvæ of the parthenogenetic generation, in the hope of finding it, and in five of them oogonial mitoses occur in which no such body is visible. In the other two no oogonia are in course of division. It is possible, of course, that all these larvæ would have developed into female-producing individuals, but since the extra-nuclear body is not recognisable in the spermatogonia in the male larva, it is more likely that if such a body is present in the female at all it is not to be found at so early a stage.

Mitoses in the Nervous System.

In a footnote added to the first part of this paper while in proof (Part I, p. 91) I mentioned that in the developing nervous system of male larvæ mitoses occurred with the haploid number (10) of chromosomes. Examples of this are shown in figs. 26, 27, 28, *a*; I have not much to add to my earlier account. I have found undoubted haploid figures in the nervous system of six male larvæ, and in no male examined are there certainly no such mitoses; in a few, no countable mitoses could be found. In addition to the haploid mitoses I have occasionally found diploid figures (figs. 28, *b*; 29); and

these appear to be in cells of a different kind. In the younger larvæ there appear to be cells of two kinds in the developing nervous system, some with numerous faint chromatic bodies in the nucleus, and a very small nucleolus, which from their appearance and distribution are probably true nerve-cells; others, more variable in size, with one or two large conspicuous nucleoli, which occur chiefly round the edge of the nervous system or in groups at a deeper level. These latter cells resemble the mesoderm cells of the larva, and the diploid mitoses appear to occur in them; possibly therefore they are immigrant mesoderm cells forming supporting tissue. In female larvæ, of both spring and summer generations, I have found only diploid mitoses in the nervous system as elsewhere.

Abnormal Nuclear Divisions in the Larva.

In the footnote referred to above, in addition to the haploid divisions in the nervous system of the male larva, I mentioned that in larvæ of both sexes giant nuclei occur below the hypodermis, some 15 or 20 μ in diameter, and that in the single mitosis which I had seen among these nuclei (an anaphase, fig. 30) there were at least 50 chromosomes at each pole. I have found no further divisions of this kind, and have made no full observations on the nature of the nuclei among which it occurs. They appear, however, to be in connection with developing muscle-fibres, between the hypodermis and the fat-body, and in younger larvæ they seem clearly to be the nuclei of the cells from which muscles are developing. The cells of the fat-body have also very large nuclei, which resemble those of the muscle cells rather closely, but usually have a less fine and regular distribution of the chromatin. Similar nuclei occur in connection with the developing muscles of the spring generation, but I have not found any in mitosis.

Summary.

The more important observations recorded are as follows:—

1. There are two kinds of parthenogenetic females in the spring generation of *Neuroterus lenticularis*, which lay eggs differing in their behaviour as regards maturation.

2. In the eggs laid by one class of female there is no maturation division; the nucleus comes to the surface, reaches the stage of prophase of division, then sinks in for a short distance and divides by a mitotic spindle parallel with the egg-margin. In such eggs early segmentation divisions show the diploid number (20) of chromosomes, and no polar chromosomes are ever found.

3. In eggs laid by the second class of female the preliminary behaviour

of the nucleus is similar to the former. Instead, however, of sinking in, it divides at the surface by a division perpendicular to the edge, producing an irregular outer group of chromosomes (first polar nucleus) and an inner group of parallel rod-like chromosomes. The latter divide immediately, apparently transversely, into an inner group which forms the egg-nucleus, and an outer or second polar group. The first polar group may divide into two. In the early segmentation mitoses of eggs of this class the haploid number (10) of chromosomes is found, and in complete series of sections of such eggs a double or triple group of polar chromosomes is always found at the edge of the egg.

4. Since it is known that some parthenogenetic individuals lay eggs which all develop into females, and others lay only male-producing eggs, and since the female shows the diploid chromosome number in all its cells, while the male has the haploid number in the spermatogonia and nerve-cells, it is suggested that the eggs which undergo no maturation division become females, those which undergo reduction males.

5. In the later segmentation stages of some eggs, and commonly in the yolk-nuclei of eggs which have reached the blastoderm stage, phenomena are described which are interpreted as indicating amitotic division.

6. The haploid mitotic figures in the nervous system of the male, referred to previously, are more fully described, and a case is mentioned of a mitosis in a developing muscle-cell with about three times the normal (diploid) number of chromosomes.

General Considerations.

The facts set forth above confirm the conclusions with regard to sex-determination in Neuroterus which were drawn provisionally in the first part of this paper. There is no need, therefore, to discuss them further. I attempted, however, in my previous discussion to link the phenomena of sex-determination in Neuroterus on to the results obtained by various methods in other groups of animals, and formulated a general scheme of sex-production, on the lines of Mendelian heredity, in which Neuroterus formed a special case. This scheme consisted in the assumption that in general the female contains female and male sex-determinants, represented by the symbols ♀ and ♂, which segregate in oogenesis so that two kinds of eggs are produced, bearing ♀ and ♂ determinants respectively. The male contains only the ♂ determinant, but is heterozygous in the sense that in its unreduced germ-cells only one ♂ determinant is present, which goes into half the spermatozoa, the other half of the spermatozoa containing no sex-determinant. This was expressed by representing the male as ♂⊙,

producing ♂-bearing and ⊙-bearing spermatozoa (the latter being those without sex-determinant). Selective fertilisation was now supposed to occur, ♀-bearing eggs being fertilised by ♂-bearing spermatozoa giving females (♀♂), ♂-bearing eggs by ⊙-bearing spermatozoa giving males (♂⊙).

It was pointed out that while such cases as *Abraxas grossulariata*, the cinnamon canary, and several breeds of fowls prove that the female is heterozygous in respect of sex, it is also clear that in some cases the male is in some sense heterozygous, as is proved by the inheritance of colour-blindness and other affections in Man. Since this was written, a new and remarkable case has been described by Morgan in Drosophila,* in which the heterozygous condition of the male is proved in exactly the same way as that of the female is shown in Abraxas. Now it is known from the work of Miss Stevens† that the male of Drosophila has a pair of unequal idio-chromosomes, and thus produces two kinds of spermatozoa, and a recent paper by Guyer‡ shows that in Man also two kinds of spermatozoa are formed. It appears, therefore, that in both the best-established cases in which the heterozygous condition as regards sex of the male has been shown by experiment and observation in heredity, the male is also heterozygous in respect of chromosomes, and produces two visibly different kinds of spermatozoa. In Abraxas, no such difference in the chromosomes of the spermatozoa is to be found,§ nor has it been found by Miss Stevens or Miss Cook in other Lepidoptera.‖ These facts naturally suggest that in some species the male is heterozygous as regards sex, in others the female, and tend to support the suggestion of Wilson,¶ Castle,** and Morgan,†† that specific male and female sex-determiners do not exist, but that femaleness consists in the presence of some extra factor superposed on what would otherwise produce maleness. If the female receives this factor from both parents, the male from only one, the female would appear homozygous as regards sex, the male heterozygous; if the female receives it from one parent and the male lacks it altogether, the female is heterozygous and the

* 'Science,' July, 1910, vol. 32, p. 120.

† 'Journ. Exp. Zool.,' vol. 5, p. 365.

‡ 'Biological Bulletin,' 1910, vol. 19, p. 219.

§ Doncaster, 'Proc. Camb. Phil. Soc.,' vol. 16, pt. 1, p. 44.

‖ N. M. Stevens, 'Carnegie Inst., Washington, Publ.,' 1906, 36, Part 2, p. 48; Cook, 'Proc. Acad. Nat. Sci., Philad.,' 1910; summarised, 'Zentralbl. f. Allg. u. Exp. Biol.,' 1910, p. 620.

¶ 'Science,' January, 1909, vol. 29, p. 53.

** 'Science,' March, 1909, vol. 29, p. 395.

†† 'Journ. Exp. Zool.,' 1909, vol. 7, p. 332. See also 'Amer. Naturalist,' vol. 45, No. 530, p. 65, which has appeared since the above was written.

male homozygous. This scheme is perfectly satisfactory as long as no case is known in which there is evidence from breeding experiments that both the male and female are heterozygous. Hagedoorn* has described such a case in fowls, but his account is not very clear and no details are given. It indicates that a heterozygous female, paired with a homozygous recessive male, transmitted the dominant character only to its male offspring; the heterozygous males so produced transmitted, in the next generation, the dominant character only to their female children. This, if correct, can only be explained on the assumption that both sexes are heterozygous for the sex-determinants, and that the dominant character is always associated in this instance with the determinant which I have represented by the symbol ♂. The hypothesis of the American writers would then fall to the ground.

Apart from this case, there is no certain means of deciding between the two hypotheses; that of Wilson, Castle, and Morgan is simpler in not requiring selective fertilisation, for which little evidence exists; but until many more cases of sex-limited inheritance are known, and it is found that they never indicate that the heterozygous condition can exist in both sexes of the same species, both hypotheses accord equally well with the known facts. One further possibility should be mentioned, which does not differ greatly from that suggested by Morgan in the paper referred to above, and also more recently by Montgomery.† This is that the "factors" which have been referred to as sex-determiners are not really such, but that every individual contains potentially the character of both sexes—is in fact potentially hermaphrodite—but for the appearance of one or other of these characters additional factors are required. If the factor which I have called the ♀ determinant is present, it brings out the female characters and suppresses the male, if the ♂ determinant is present in the absence of the ♀, it brings out the male characters. On such a hypothesis as this the results arrived at by Geoffrey Smith‡ would be explained. He infers a "sexual formative substance" in Malacostracan Crustacea, which causes the appearance of female characters; when a male is parasitised by Sacculina the parasite induces the secretion of such a substance by the organism, and the animal assumes the female characters and may even produce eggs in its testis. Even though such a male might, on the hypothesis summarised above, have the constitution ♂ ⊙, *i.e.* might not contain the factor which brings out the female characters, yet the secretion induced by the parasite might have the same effect as this factor, and thus cause the

* 'Archiv f. Entwicklungsmechanik,' 1909, vol. 28, pp. 18, 26.

† 'Biological Bulletin,' 1910, vol. 19, p. 9.

‡ "Studies in the Experimental Analysis of Sex," 'Q.J.M.S.,' 1910, vol. 55, p. 225.

female characters to appear. This hypothesis of potential hermaphroditism of both sexes, combined with special ♂ and ♀ activators, would thus allow of the occasional modification of sex by environment, if the stimuli supplied were able to bring about the same physiological effects as the inherited ♂ and ♀ determinants. That environment may have some such effect in modifying somatic characters which are inherited alternatively is indicated by the work of Tower,* and there seems to be no *a priori* reason for denying such an effect in the case of sex.

EXPLANATION OF FIGURES ON PLATE 17.

All the figure are freehand drawings, made with a Zeiss aprochromat. 3 mm., 1·40 n.a., and compensating ocular 12.)

Figs. 1—3.—Three stages of the approach of the nucleus to the edge of the egg, preceding maturation.

Figs. 4, 5.—Prophases of first segmentation division; diploid type of egg.

Fig. 6 (*a*, *b*).—First segmentation division, same series as figs. 4, 5; *a* and *b* show the mitotic figure cut in two successive sections.

Fig. 7.—Completion of first segmentation division; same series as figs. 4—6.

Fig. 8.—Anaphase group of segmentation mitosis, diploid type. Nineteen chromosomes are visible in this section; at the other end of the same spindle, two sections removed, 15 or 16 can be counted.

Fig. 9.—Prophase of diploid segmentation division, showing about 20 chromosomes arranged in irregular meridional bands under the nuclear membrane.

Fig. 10.—Late prophase, same series as fig. 8, showing intranuclear origin of spindle.

Fig. 11.—Prophase of polar division, haploid type of egg.

Fig. 12.—First polar division, nearly completed. The division figure ran obliquely through two sections; all the inner part is drawn from one section, the greater part of the outer group of chromosomes from the next.

Fig. 13 (*a*, *b*).—Second polar division; the division figure runs obliquely through two sections. *a* represents the separation of the chromosomes of the egg-nucleus from the second polar chromosomes; *b*, in the next section, the outer polar group.

Fig. 14 (*a*, *b*).—Two successive sections, showing completion of second polar division. In *a*, egg-nucleus on right, second polar group in middle, part of division spindle of outer polar group on left; *b*, division-figure of outer (first polar) group.

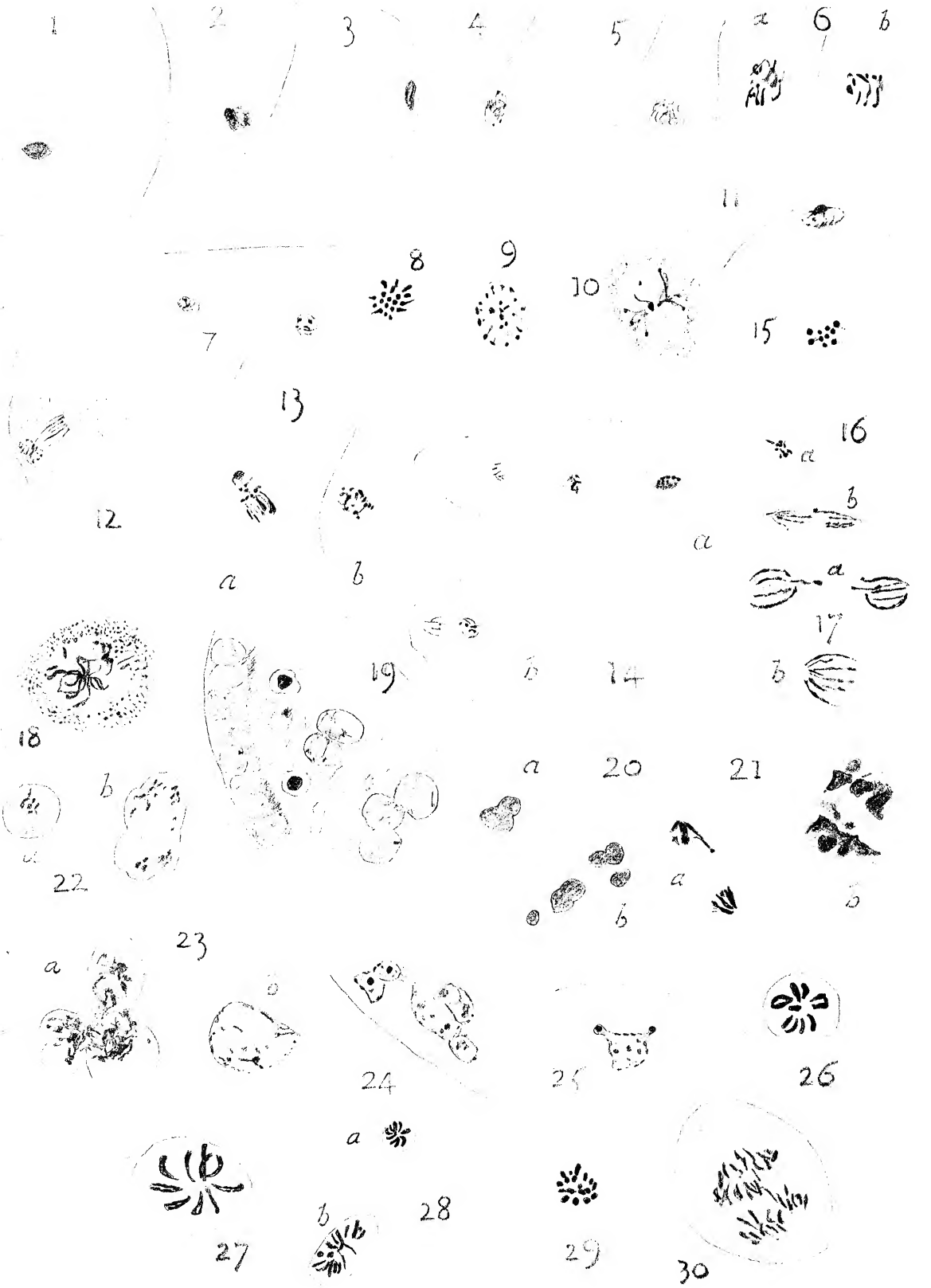
Fig. 15.—Anaphase group of segmentation division, haploid type.

Fig. 16 (*a*, *b*).—Anaphases of haploid segmentation divisions; *a*, cut across; *b*, seen sideways. Both from the same egg.

Fig. 17 (*a*, *b*).—Two late anaphases, haploid type, from the same egg. In *a* only the chromosomes at the upper side of the figure are shown. In *b* all the chromosomes (8) visible in the section are shown; the other end of the figure was broken.

Fig. 18.—Group of polar chromosomes during segmentation stages; same egg as fig. 15. To the left, a large group of about 20 (outer polar group); to the right, smaller inner polar group.

* 'Biological Bulletin,' 1910, vol. 18, p. 285.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b
23
a
b
24
25
26
a
27
b
28
29
30

FIG. 19.—Part of an egg in blastoderm stage, showing two groups of vesicular nuclei apparently undergoing amitotic division and two smaller nuclei with dark mass in the centre.

FIG. 20 (*a*, *b*).—Stages of apparent amitotic division in blastoderm stage. *a*, early stage; *b*, a group of nuclei from another part of the same section.

FIG. 21 (*a*, *b*).—Division-figures in an abnormal egg. *a*, division of smaller nucleus suggesting haploid type; *b*, division of larger nucleus in the same egg.

FIG. 22 (*a*, *b*).—Nuclei in an egg from same series as fig. 21. *a*, ordinary nucleus; *b*, large nucleus undergoing amitotic division or fusion.

FIG. 23 (*a*, *b*).—*a*, amitotic division or compound fusion; *b*, single nucleus from same egg. Same series as figs. 21, 22.

FIG. 24.—Group of nuclei (amitosis or fusion). The two nuclei to the left are drawn from the next section to the larger group. Same series as fig. 20.

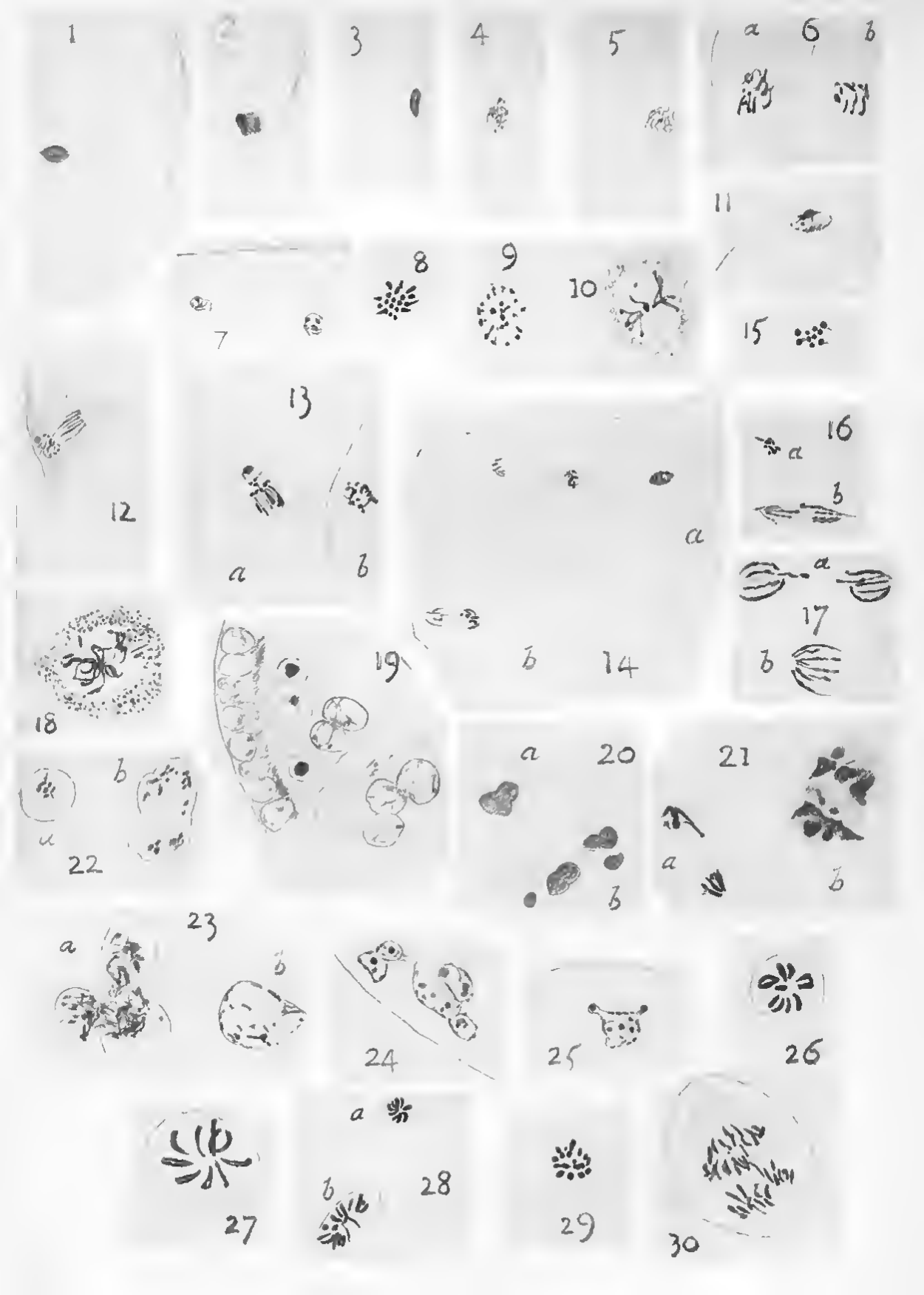
FIG. 25.—Nucleus showing irregular shape. Same egg as fig. 24.

FIGS. 26, 27.—Haploid divisions (metaphase) in nervous system of male larvæ (summer generation). In fig. 27 each chromosome is seen split preparatory to division.

FIG. 28 (*a*, *b*).—Two equatorial plates in the nervous system of male larva. *a* is haploid (9, or possibly 10, chromosomes visible); *b*, diploid (about 16 visible).

FIG. 29.—Diploid group, nervous system of male larva (same specimen as fig. 28). Eighteen chromosomes are visible.

FIG. 30.—Mitosis of giant nucleus in muscle-cell, female larva, summer generation. The figure extends through two sections, only one of which is represented. Less than half of the chromosomes are shown.


1
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4
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a
6
b
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12
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17
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